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# Bioavailability of iron complexed with organic colloids to the cyanobacteria *Synechococcus* and *Trichodesmium*

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ABSTRACT: Iron (Fe) is an important limiting micronutrient to phytoplankton productivity in several major oceans due to its very low dissolved concentration and dominance by organic complexation. The biological uptake of Fe complexed with natural colloidal nanoparticles (measuring between 1 kDa and 0.2 µm), which are abundant in natural seawater, is important for the understanding of Fe limitation in the sea. In this study, we quantified the bioavailability of Fe complexed with colloids of various geochemical properties to 2 important marine cyanobacteria, *Synechococcus* and *Trichodesmium*. We demonstrated that the uptake of colloidal Fe was greatly dependent on the origin, size, aging, and Fe concentration of the colloids. The uptake of colloidal Fe was at least 1.7× lower than the uptake of Fe complexed with low molecular weight compounds (<1 kDa). Fe bound with colloids isolated from estuarine waters was bioavailable to 2 cyanobacteria, whereas Fe bound with coastal colloids was little bioavailable to the cyanobacteria. Furthermore, colloidal Fe resulting from *Synechococcus* decomposition was also accumulated by *Synechococcus*, whereas colloidal Fe from diatom *Thalassiosira pseudonana* decomposition was unavailable to them. In general, smaller sized colloids (1 to 10 kDa) and shorter aging of colloids enhanced Fe uptake by the 2 cyanobacteria. These results indicate that dissociation of Fe from colloidal particles may be important in controlling Fe bioaccumulation by the cyanobacteria. At typical natural colloidal Fe concentrations, the calculated uptake rate-constant of Fe was relatively independent of the Fe concentration, whereas at a much higher colloidal Fe concentration (>549 nM), the uptake rate constant decreased significantly. Overall, our data demonstrated that natural colloidal Fe is bioavailable to marine cyanobacteria, but the bioavailability is clearly dependent on the colloidal geochemical characteristics.

KEY WORDS: Bioavailability · Colloids · Iron · Cyanobacteria



## INTRODUCTION

Over the past decades, substantial evidence has accumulated to indicate that marine colloidal nanoparticles, defined as the size fraction between 1 kDa and 0.2 µm, are abundant in natural seawater (Buffle et al. 1995, Guo & Santschi 1997). These small nanoparticles can bind strongly with trace metals, and thus may form a major potential metal-sink. Whether these colloidal nanoparticles are actively involved in the biological cycling of trace metals has not yet been extensively addressed (Wang & Guo 2000). The fractions of metals bound with the colloidal particles are metal-specific, and depend on geochemical environments (Martin et al. 1995, Guo & Santschi 1997, Santschi et al. 1999). Over the past few years, several studies have suggested that most of the traditionally defined dissolved Fe (<0.2 µm) are in fact associated with the colloidal particles, particularly with the relatively large size of colloids (Santschi et al. 1999, Wen et al. 1999, Wells et al. 2000, Wu et al. 2001). Colloidal particles have been shown to be the most dynamic fraction of dissolved metals during phytoplankton growth in both laboratory and field studies (Nishioka & Takeda 2000, Kuma et al. 2000a).

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Iron is biologically essential to marine phytoplankton, as it is actively involved in algal photosynthesis, electron transport, and nitrate and nitrite metabolism (e.g. nitrate and nitrite reductase) (Geider & LaRoche 1994). The effect of Fe limitation on marine phytoplankton productivity in the sea has been well documented (Martin & Fitzwater 1988, Martin et al. 1994, Hutchins et al. 1998, Boyd et al. 2000). One major attribute underlying Fe limitation on phytoplankton growth is the extremely low dissolved Fe concentration in these regions, as well as the low solubility of Fe in seawater (Liu & Millero 2002). Furthermore, Fe is mostly (>98%) complexed with organic ligands, and the resulting inorganic Fe concentration, which is thought to be the bioavailable fraction, is extremely low (e.g. subnanomolar levels, Rue & Bruland 1995, 1997, Wu & Luther 1995). Siderophore (molecular size of 300 to 1000 Da), produced by marine cyanobacteria, is an important ligand binding with Fe in natural seawater (Macrellis et al. 2001).

Because of the significance of biological uptake of Fe, and the dominance of Fe complexation in natural seawater, several studies have examined the uptake by marine phytoplankton of Fe complexed with a variety of organic ligands (e.g. Kuma & Matsunaga 1995, Kuma et al. 2000b, Maldonado & Price 1999, Soria-Dengg et al. 2001). Many studies have generally used well-characterized siderophore-like compounds to examine the bioavailability to marine phytoplankton (Hutchins et al. 1999, Maldonado & Price 1999, 2000, Boye & van den Berg 2000, Kuma et al. 2000a). Conflicting conclusions are drawn from these limited studies. A few studies have shown that marine phytoplankton are able to take up such organically complexed Fe (Soria-Dengg & Horstmann 1995, Hutchins et al. 1999, Maldonado & Price 1999, 2000, Kuma et al. 2000b, Soria-Dengg et al. 2001), whereas other studies found that organically complexed Fe was not bioavailable to phytoplankton (Wells 1999, Boye & van den Berg 2000). In contrast, very few studies have considered the biological uptake of colloidal bound Fe to marine phytoplankton (Wells et al. 1983, 1991a, Rich & Morel 1990, Wells & Mayer 1991, Kuma & Matsunaga 1995, Nodwell & Price 2001), and even fewer studies considered the uptake of Fe bound with natural colloids (Chen & Wang 2001, Chen et al. 2003). Whereas many studies used siderophore-like compounds to examine Fe uptake, the significance of these compounds in sequestering Fe in natural seawater remains to be further revealed (Macrellis et al. 2001). Siderophores typically have a molecular size <1000 Da (Trick & Kerry 1992, Macrellis et al. 2001), and are presumably excluded from the traditionally defined colloidal size. Given the importance of colloidal particles in natural seawater, there is clearly a substantial need to directly examine the biological availability of colloidal bound Fe to marine phytoplankton.

In a recent study, we quantified the biological availability of Fe bound with natural colloids to a marine diatom *Thalassiosira pseudonana* (Chen & Wang 2001). We found that Fe bound with natural colloids was indeed biologically available to the diatoms, but the bioavailability depended on various geochemical properties of the natural colloids (Chen & Wang 2001). Hutchins et al. (1999) provided experimental evidence on the difference in bioavailability of organically complexed Fe to marine prokaryotes and eukaryotes. There was clear ecological separation between these 2 groups of phytoplankton in sequestering Fe. This study, therefore, examined the biological availability of colloidal Fe by 2 marine cyanobacteria. These organisms have an efficient siderophore-based system to scavenge Fe under Fe-limited conditions (Trick 1989, Ferreira & Straus 1994, Wilhelm & Trick 1995, Wilhelm et al. 1998), thus forming an important phytoplankton community in Fe-limited environments. Several species of cyanobacteria (e.g. from the genera *Synechococcus* and *Trichodesmium*) are also able to induce IdiA protein–homologues, thus facilitating Fe scavenging (Webb et al. 2001). Furthermore, Fe availability may be a limiting factor to nitrogen fixation in cyanobacteria (Paerl et al. 1994, Berman-Frank et al. 2001), although recent studies also demonstrated that phosphate is limiting to nitrogen fixation in the open oceans (Wu et al. 2000, Sanudo-Wilhelmy et al. 2001). We considered various geochemical properties of the colloids (colloidal organic-carbon concentration, size, origin, aging, and colloidal Fe concentration) in this study, and quantified the biological availability of colloidal Fe by measurements of intracellular Fe accumulation using a radiotracer spiking technique. No previous study has quantified the uptake of natural colloid-bound Fe to marine cyanobacteria.

## MATERIALS AND METHODS

**Cyanobacteria.** *Synechococcus* spp. (CCMP 1333) was purchased from the Provasoli-Guillard Phytoplankton Collection Center and maintained in f/2 medium (Guillard & Ryther 1962) in axenic culture at 18°C. *Synechococcus* is an important picoplankton in oceanic waters (Burkill et al. 1993, DuRand et al. 2001). *Trichodesmium* spp. was kindly provided by Dr. S. Lin from the University of Connecticut, USA, and was maintained in axenic culture in the medium as described in Chen et al. (1996). *Trichodesmium* is also a dominant phytoplankton in tropical and subtropical oceans (Carpenter et al. 1999, Chang et al. 2000). All experiments used exponentially growing

cells (Fe-repleted) and were conducted at 18°C with a light illumination of 70 µmol $m^{-2}$ $s^{-1}$, except when specified below for dark conditions.

**Isolation of colloids and radiolabeling.** Seawater was collected from 3 different locations in Hong Kong (Yuen Long, salinity of 12 psu; Tolo Harbor, salinity of 30 psu; Clear Water Bay, salinity of 30 psu). Yuen Long is close to the mouth of the Pearl River Estuary and under heavy influence of river discharge from the estuary, especially during the summer seasons. Tolo Harbor is an eutrophied semi-enclosed bay, with considerable domestic sewage discharge from the harbor area. Phytoplankton production in the harbor is high throughout the year. Clear Water Bay is on the eastern side of Hong Kong, with a significant influence from the South China Sea. The water can be considered as oligotrophic, with notable oceanic origins. The seawater was immediately processed upon arrival to the laboratory. The background dissolved Fe concentrations, as measured by ICP-MS with Dynamic Reaction Cell (DRC) (Shikino et al. 2001), were 50, 59, and 55 nM in Yuen Long, Tolo Harbor, and Clear Water Bay, respectively. Dissolved organic carbon concentrations in the seawater were 86 to 126 µM, 117 to 201 µM, and 98 to 130 µM in Yuen Long, Tolo Harbor, and Clear Water Bay, respectively. Colloids were collected using our established methods (Wang & Guo 2000, Chen & Wang 2001). Briefly, the seawater was first passed through the glass fibre filter and then a 0.22 µm Millipore cartridge. The filtered seawater was subsequently ultrafiltered by a spiral-wound cross-flow ultrafiltration cartridge (Amicon), with a 1 or 10 kDa cutoff (Guo & Santschi 1996, Guo et al. 2000, Wang & Guo 2000), using a concentration factor of 40. Colloidal collection was conducted in an enclosed system (with acid clean Telfon tubing) to minimize contamination of the seawater. The permeate (<1 kDa, low molecular weight, LMW) resulting from ultrafiltation of Clear Water Bay seawater was used in all radiolabeling and uptake experiments.

The colloids were immediately radiolabeled with $^{59}$Fe (obtained from New England Nuclear, in 0.1 N HCl), using methods described in Chen & Wang (2001). $^{59}$Fe (296 kBq, corresponding to 122 nM) was spiked into 70 ml of colloids in a Telfon bottle. After radiolabeling (described below), the colloids were placed in a dialysis bag (1 or 10 kDa cutoff) suspended in 2 l LMW seawater for 2 d. The LMW water was replaced every 12 h to remove any unlabeled Fe. The $^{59}$Fe activity in the LMW water after 2 d of dialysis was essentially undetectable (<3 pM), thus most $^{59}$Fe was complexed with the colloids within the dialysis bag. Radiolabeling efficiency in these experiments was generally high (>75%). After dialysis, the radiolabeled colloids were filtered through 0.22 µm polycarbonate membrane to again remove large particles formed by coagulation, and immediately used for the experiments. The resulting $^{59}$Fe concentrations in the uptake medium (after a 40× dilution of the radiolabeled concentrated colloids) were 6.3 to 7.0 nM, which were much lower than the background dissolved Fe concentration (50 to 59 nM). $^{59}$Fe in the sample was measured by a Wallac 1480 gamma detector at 1092 keV.

**General procedures of measuring colloid-bound Fe uptake.** Cyanobacteria in exponential growth phase were removed by filtration and resuspended in 150 ml LMW seawater held in an acid-cleaned polycarbonate bottle. All manipulation was performed in a trace metal class-100 bench to minimize potential Fe contamination of the samples. The initial density of the cells was 2 to $3 \times 10^6$ cells $ml^{-1}$ for *Synechococcus*, and 1.3 to $2.5 \times 10^5$ cells $ml^{-1}$ for *Trichodesmium*. A control treatment without the addition of phytoplankton cells but with the radiolabeled colloids was also prepared to monitor the partitioning of colloidal Fe during the course of the experiments. Each treatment contained 3 replicated bottles. In addition, each experiment had one LMW complexed Fe treatment. To obtain Fe in the LMW complexed form, $^{59}$Fe was first added to the colloids contained in a dialysis bag (with 1 kDa cutoff) and the pH was adjusted to 8.0. Any uncomplexed $^{59}$Fe was separated by the dialysis bag into the surrounding LMW seawater, which was collected after 12 h of equilibrium. The detailed species of Fe in the LMW fraction (<1 kDa) was, however, unknown. This fraction was subsequently added into LMW seawater, resulting in a $^{59}$Fe concentration of 6.0 to 8.6 nM before the uptake experiments.

The cells were incubated at a light illumination of 70 µmol $m^{-2}$ $s^{-1}$. At 2, 4, 6, and 8 h of exposure, the cells were taken for cell density measurements (under the microscope for *Synechococcus* or fluorometer measurements for *Trichodesmium*). A 2 ml aliquot of sample was taken for measurement of total $^{59}$Fe radioactivity in the water. A 10 to 20 ml sample was filtered onto a 1 µm polycarbonate membrane. The extracellular bound Fe was removed by Ti-EDTA-citrate regents (Hudson & Morel 1989) for 2 min, and the membrane was further rinsed with LMW seawater. The filter was measured for radioactivity, representing the intracellular accumulated $^{59}$Fe. After 8 h of the exposure, the total cellular radioactivity of $^{59}$Fe (intracellular and extracellular) in the cells was also measured by filtering a 20 ml sample onto the polycarbonate membrane. In our study, Fe internalization fluxes ($Q_m$) can be calculated as:

$$Q_m = k_u \times C_{bulk} \quad (1)$$

where $k_u$ is the uptake rate constant for complexed Fe, and $C_{bulk}$ is the Fe concentration in the solution. Since

the background colloidal Fe concentration was not quantified, the $C_{bulk}$ (including the background Fe and the spiked $^{59}$Fe) was not known. Furthermore, there were some differences in the total colloidal Fe concentrations among different experimental treatments (colloidal sizes, origins, and colloidal organic carbon concentrations), thus the uptake rate ($Q_m$) cannot be directly compared among the treatments. We therefore calculated the uptake of colloidal Fe using the concentration factor in the algal cells (CF, l kg$^{-1}$), by the following equation (Chen & Wang 2001):

$$CF = \frac{C_p}{C_w} \quad (2)$$

where $C_p$ is the radioactivity in the intracellular cells (ccpm kg$^{-1}$ dry wt), and $C_w$ is the radioactivity in seawater (ccpm l$^{-1}$). The CF can be considered as the relative uptake of Fe by the cells. The cell dry weight was measured after filtering a known volume of water onto glass fiber filters and then rinsing with ammonium formate and drying at 80°C overnight. Because uptake took place following a linear pattern (see 'Results'), the slope of the linear regression between CF and the time of exposure (2 to 8 h) represented the uptake rate constant $k_u$ (or uptake coefficient), which can be compared among different experimental treatments. The comparison of $k_u$ among different treatments was preceded by the assumption that it is relatively independent of total colloidal Fe concentrations, which was also specifically tested in this study (see below). The absolute metal uptake rate, or internalization flux ($Q_m$), can be calculated as $k_u \times C_{bulk}$ using Eq. (1). The percentage of total cellular Fe in the intracellular pool (i.e. the intracellular Fe distribution) was calculated as the radioactivity in the intracellular pool of cells (measured after Ti reagent washing) divided by the total $^{59}$Fe radioactivity in the whole cells, measured at 8 h exposure.

The partitioning of radiolabeled colloid-bound Fe in the medium was quantified by a stirrer cell with a 1 kDa ultrafiltration membrane (Amicon YM1, regenerated cellulose) or a centrifugal ultrafilter with a molecular weight cutoff of 10 kDa, as described in Chen & Wang (2001).

**Influences of colloidal origins and colloidal organic carbon (COC) concentrations on Fe uptake.** Colloids isolated from 2 contrasting environments were used in this experiment, including Yuen Long (representing the estuarine environments with a typical salinity of 12 psu, and Clear Water Bay, representing oligotrophic coastal environments with a salinity of 30 psu and significant influence of oceanic currents). The estuarine colloids were radiolabeled with $^{59}$Fe in LMW water isolated from Clear Water Bay. Any coagulated particles were removed by filtering the water onto the 0.22 µm polycarbonate membrane after radiolabeling and dialysis. Two different COC concentrations were used in experiments with *Synechococcus*. The lower COC concentration was the same as natural COC concentrations in the field (17 to 28 µM, designated 1C), and the higher COC concentration was 3× higher than background COC concentration in natural seawater (51 to 84 µM, designated 3C). Only 1C was used in the experiment with *Trichodesmium*.

In a separate experiment, we compared the uptake of Fe bound with colloids originated from decompositions of phytoplankton cells. Two phytoplankton were used in this experiment, including the diatom *Thalassiosira pseudonana* (Clone 3H) and *Synechococcus*. The 2 algal species were radiolabeled with $^{59}$Fe, as described in Wang & Guo (2001). Briefly, algal cells in the exponential growing phase were filtered and resuspended in 0.2 µm filtered seawater enriched with f/2 levels of N, P, Si, and vitamins, and f/20 levels of trace metals without EDTA, Cu and Zn, and finally $^{59}$Fe. After 3 d growth for diatoms and 6 d growth for *Synechococcus*, the cells divided several times and were thus uniformly radiolabeled with $^{59}$Fe. They were collected by filtration and resuspended into LMW seawater (without nutrient additions). The labeled cells were subsequently placed in the dark and allowed to decompose for 4 wk (Wang & Guo 2001), during which period they were regularly shaken. After decomposition, the water was filtered through a 0.2 µm filter and the detritus was discarded. The filtrate was added to the dialysis bag suspended in LMW water stirred with a magnetic bar. The $^{59}$Fe in the LMW fraction was removed by renewing the LMW water every 12 h. After 2 d dialysis, when there was minimal $^{59}$Fe detected in the LMW water (thus all the $^{59}$Fe was bound with the decomposed colloids), the radiolabeled colloids were collected and the colloidal Fe uptake was compared with the uptake of the LMW-bound Fe, using methods described above.

**Influences of colloidal size and light condition on Fe uptake.** Two different colloid sizes were considered in this study: 1 to 10 kDa and 10 kDa to 0.2 µm. Colloids were isolated by 1 or 10 kDa ultrafiltration cartridges using a concentration factor of 40 for each ultrafiltration step. Colloids isolated from Yuen Long and Tolo Harbor were used in experiments with *Synechococcus*. Furthermore, the uptake of 2 colloidal sizes isolated from 2 different systems under light and dark conditions was compared. For *Trichodesmium*, we only compared the uptake of 2 colloidal sizes from Yuen Long. The uptake of colloidal Fe under light and dark was contrasted in a separate experiment.

**Influences of colloidal aging on Fe uptake.** Colloids isolated from Yuen Long were used in this experiment. Choice of Yuen Long colloids was based on the relatively higher uptake of colloidal Fe by the cyanobacteria than

from the other 2 locations. The colloids were radiolabeled for 1 and 12 d, respectively; afterwards, they were collected as described above. The radiolabeled colloids were then distributed to the exposed medium, and the uptake was compared. In this experiment, the colloids were radiolabeled at different times, such that the uptake of the 2 differently aged colloids were conducted simultaneously using the same batch of algae. We only examined the uptake by *Synechococcus* in this experiment.

**Influences of colloidal Fe concentration on Fe uptake.** This experiment examined the influences of different colloidal Fe concentrations on Fe uptake by *Synechococcus*. The colloids (1 kDa to 0.2 µm) were collected from Yuen Long as described, and were spiked with different nominal concentrations (0, 2, 8, 32, 50 µM) of Fe (in $FeCl_3$) with the presence of $^{59}Fe$ radioisotope. After 2 d, the radiolabeled Fe at different concentrations was placed in a dialysis bag and any uncomplexed Fe was removed by dialysis. The radiolabeled colloidal Fe was then quantified for its uptake by *Synechococcus*. The resulting nominal Fe concentrations in the radiolabeled colloids during the uptake experiments was 38, 81, 159, 549, and 777 nM, respectively.

## RESULTS

### Fe uptake at different COC concentrations and from different origins

In all experiments, the majority of the colloidal Fe (>93%) remained in the colloidal phase in the controlled treatments without phytoplankton additions. Uptake of colloidal Fe and LMW complexed Fe (<1 kDa) proceeded linearly between 2 and 8 h of exposure for both species of cyanobacteria, except for the LMW complexed Fe uptake by *Trichodesmium*, which appeared to reach saturation after 6 h of exposure (Fig. 1). Two COC concentrations (1× and 3× background COC) were examined for *Synechococcus*, whereas only the background COC concentration was examined for *Trichodesmium*. The uptake of LMW-bound Fe was much higher than the uptake of colloid-bound Fe. By the end of 8 h exposure, the calculated concentration factor (CF) in *Synechococcus* was 3.7 to 4.2× and 27.5 to 34.9× higher than Fe bound with Yuen Long (YL) and Clear Water Bay (CWB) colloids, respectively. The CF in *Trichodesmium* was 1.3 and 10.8× higher than Fe bound with YL and CWB colloids, respectively. Between the 2 types of colloids, the CFs of YL colloids were much higher (7.4 to 8.3× for *Synechococcus* and 8.2× for *Trichodesmium*) than those of the CWB colloids. Fe bound with the coastal colloids was barely accumulated by the 2 species of cyanobacteria. No major difference was found between the background and 3× COC treatments for *Synechococcus* for colloids isolated from the 2 different systems.

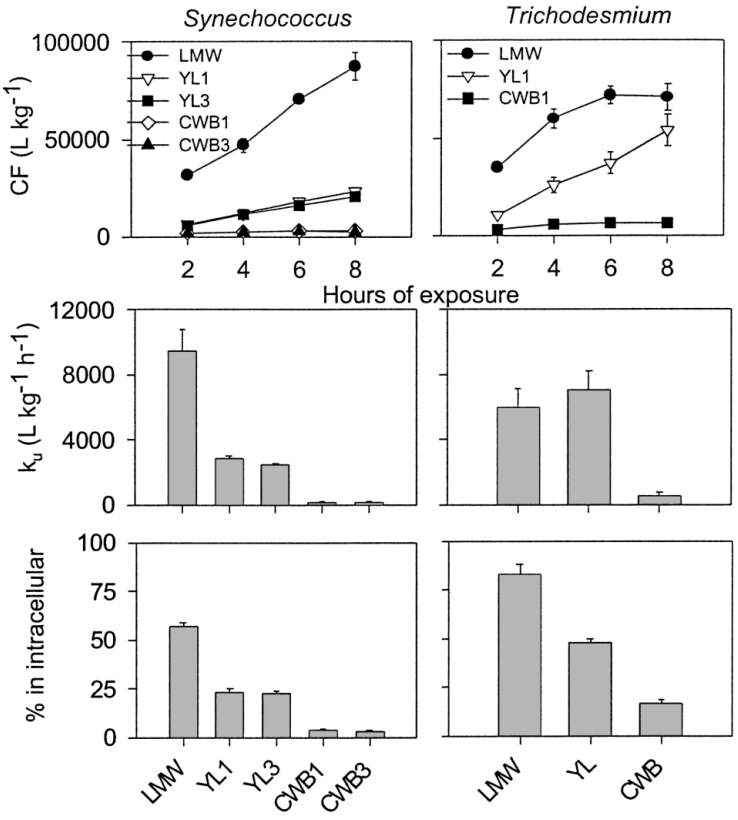


Fig. 1. Concentration factor (CF), uptake rate constant ($k_u$), and % in intracellular pool, of $^{59}Fe$ bound with natural colloids isolated from estuarine (Yuen Long, YL) and coastal (Clear Water Bay, CWB) waters in 2 cyanobacteria: *Synechococcus* (left panel) and *Trichodesmium* (right panel). LMW: Fe complexed with low molecular weight fraction (<1 kDa), YL1: Yuen Long colloid at background colloidal organic carbon (COC) concentration, YL3: Yuen Long colloids at 3× background COC concentration, CWB1: Clear Water Bay colloid at background COC concentration, CWB3: Clear Water Bay colloids at 3× background COC concentration. Values are means ± SD (n = 3)

The uptake rate constant ($k_u$) was calculated as the slope of the linear regression between the CF and the time of exposure (2 to 8 h) (Fig. 1). The intercept of the regression can be considered as adsorbed Fe that could not possibly be removed by Ti reagent washing (which removed the extracellular fraction of Fe). Uptake within the first 2 h was not included in our analysis, since the significant uptake might also include the Fe adsorption. The $k_u$ of LMW-bound Fe in *Synechococcus* was 3.3 to 3.8× and 50 to 60× higher than the $k_u$ of YL and CWB colloid-bound Fe ($p < 0.001$, *t*-test). In contrast, the $k_u$ of LMW-bound Fe in *Trichodesmium* was comparable to that of colloids isolated

from YL ($p > 0.05$, *t*-test), but was 11× higher than the $k_u$ of colloids isolated from CWB. The $k_u$ of YL colloid-bound Fe was also significantly higher (14.9 to 17.2× for *Synechococcus* and 13.1× for *Trichodesmium*, respectively) than the $k_u$ of CWB colloid-bound Fe ($p < 0.001$, *t*-test). A similar trend was also observed in the distribution of total cellular Fe in the intracellular compartment after 8 h exposure (Fig. 1). Approx. 57 and 83% of total cellular Fe was found in the intracellular pool in the LMW treatment for *Synechococcus* and *Trichodesmium*, respectively. In contrast, the intracellular distribution was much smaller in the colloidal treatments. Only 23 and 48% of total cellular Fe was in the intracellular pool in the YL colloidal treatment for *Synechococcus* and *Trichodesmium*, respectively. For the coastal colloidal treatment, the fractions were much smaller than the YL colloidal treatment, i.e. 4 and 17%, respectively.

Uptake of biologically originated colloids (by phytoplankton decomposition) by *Synechococcus* is shown in Fig. 2. A similar pattern of linear uptake was also documented in this experiment. There was a major difference among the different experimental treatments (LMW, colloids obtained from *Synechococcus* decomposition, and colloids obtained from diatom *Trichodesmium pseudonana* decomposition). Very little increase in CF was evident for the diatom colloidal treatment during the course of exposure. After 8 h exposure, the calculated CF of LMW treatment was 1.8× and 6.4× higher than the CF in the *Synechococcus* and diatom colloidal treatment, respectively. The $k_u$ of LMW complexed Fe was 1.7× and 18.5× higher than the $k_u$ of *Synechococcus* and diatom colloidal treatments ($p < 0.01$, *t*-test). A much smaller fraction of Fe was detected in the intracellular pool in the diatom colloidal treatment as compared to the other 2 treatments (Fig. 2).

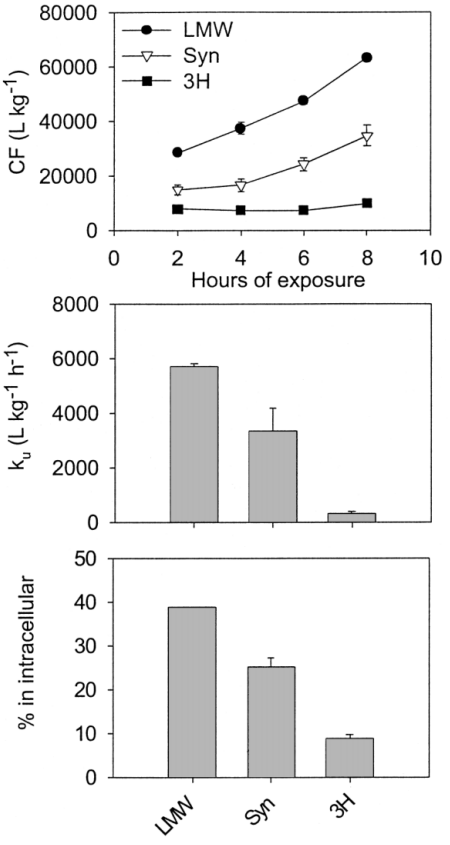


Fig. 2. Concentration factor (CF), uptake rate constant ($k_u$), and % in intracellular pool, of $^{59}$Fe bound with colloids from phytoplankton decomposition in *Synechococcus*. LMW: Fe complexed with low molecular weight fraction (<1 kDa), Syn: *Synechococcus* decomposed colloids, 3H: diatom *Thalassiosira pseudonana* decomposed colloids. Values are means ± SD (n = 3)

### Fe uptake from different sizes of colloids and under light and dark conditions

In general, a linear pattern of uptake by *Synechococcus* occurred between 2 and 8 h, except for the LMW treatment under light conditions, in which the uptake appeared to reach saturation after 4 h of exposure (Fig. 3). The calculated CF differed between the LWM treatment and colloidal treatments, especially under light conditions. At 8 h, the difference in CF between the LMW treatment and the Tolo Harbor (TH) larger colloidal treatment (which had the lowest CF) was about 9.6× under light and 5.6× under dark conditions. The CF of LMW treatment under light conditions was 4.3× higher than that under dark conditions after 8 h. The calculated $k_u$ of LMW Fe was significantly higher than all the colloidal treatments ($p < 0.05$, *t*-test). Among the different colloidal treatments, colloids isolated from YL had a higher $k_u$ than colloids isolated from TH in both light and dark experiments. When the 2 different colloidal sizes were compared (1 to 10 kDa and 10 kDa to 0.2 µm), the $k_u$ of the smaller colloidal treatment from YL was 2.1× higher than the $k_u$ of larger-sized colloids under light conditions, whereas the $k_u$ from TH was comparable between the 2 colloidal size treatments. There was very little difference between the 2 colloidal size treatments for both YL and TH colloids under dark conditions. Similarly, the intracellular distribution of the YL colloids was higher for the smaller (6.9%) than for the larger colloidal treatment (4.5%) under light conditions. No difference was found for the differently sized colloidal treatments under dark conditions.

Fe uptake by *Trichodesmium* of 2 different sizes of colloids isolated from YL is shown in Fig. 4. The CF was 2.6 to 3.2× higher in the LMW treatment than in

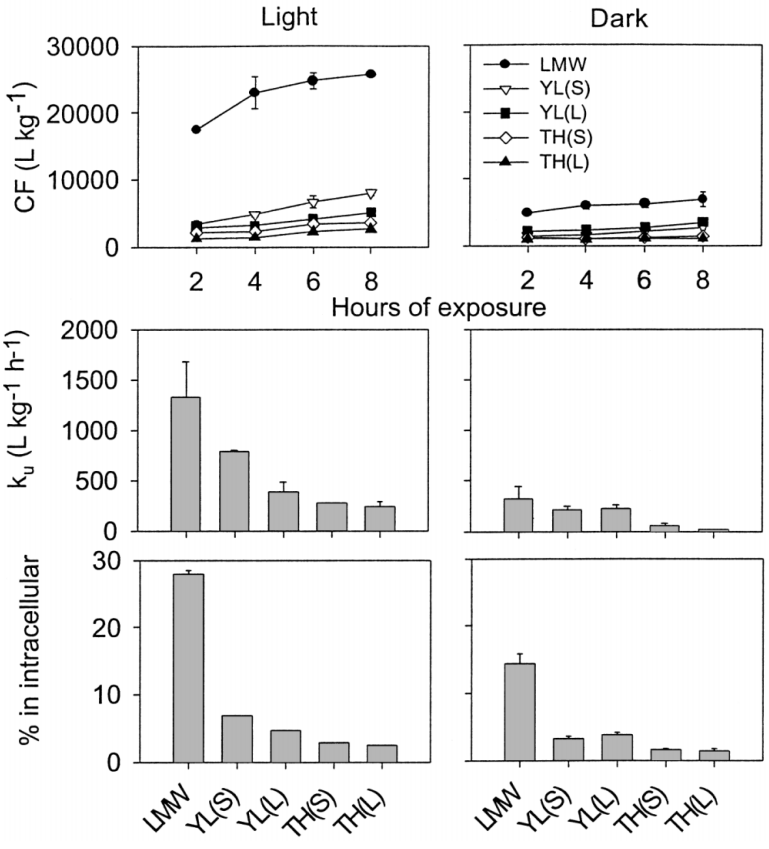


Fig. 3. Concentration factor (CF), uptake rate constant ($k_u$), and % in intracellular pool, of $^{59}$Fe bound with different sizes of natural colloids isolated from estuarine (Yuen Long, YL) and harbor (Tolo Harbor, TH) waters in *Synechococcus*, under light (left panel) and dark conditions (right panel). LMW: Fe complexed with low molecular weight fraction (<1 kDa), YL(S): Yuen Long small colloid (1 to 10 kDa), YL(L): Yuen Long large colloids (10 kDa to 0.2 µm), TH(S): Tolo Harbor small colloids (1 to 10 kDa), TH(L): Tolo Harbor large colloids (10 kDa to 0.2 µm). Values are means ± SD (n = 3)

the colloidal treatments. There was no significant difference between the calculated $k_u$ of the 2 different colloidal size treatments ($p > 0.05$, *t*-test), although it was 1.4× higher for the smaller than for the larger colloidal treatment. The intracellular distributions among the different experimental treatments were comparable. Approx. 47 to 61% of total cellular Fe was distributed in the intracellular pool of the cells in this experiment.

After 8 h of exposure, the CF of LMW Fe in *Trichodesmium* under light conditions was 6.2 to 9.8× higher than the CFs of the colloidal treatments under light and dark conditions (Fig. 5). In this experiment, the CF and $k_u$ of the colloidal treatment in the light was 1.6 and 1.7×, respectively, higher than those in the dark. There was no major difference in the intracellular distribution of the cells among the different treatments (41 to 53%).

### Fe uptake from colloids of different ages

Colloids were radiolabeled with $^{59}$Fe for 1 and 12 d, and their uptake by *Synechococcus* was compared (Fig. 6). The CF after 8 h of exposure was 2.3 to 3.0× higher in the LMW treatment than the colloidal treatments, and the CF in the 1 d old colloidal treatment was 1.3× higher than the 12 d old colloidal treatment. The $k_u$ was 1.4× higher for colloids aged 1 d than for colloids aged 12 d ($p < 0.05$, *t*-test), implying that the older colloids had a lower uptake than the younger colloids. The $k_u$ of LMW Fe was 1.8× higher than the $k_u$ of the 1 d old colloidal treatment. There was no major difference between the 2 colloidal treatments in their Fe distributions in the intracellular pool, but they were lower than the LMW treatment (Fig. 6).

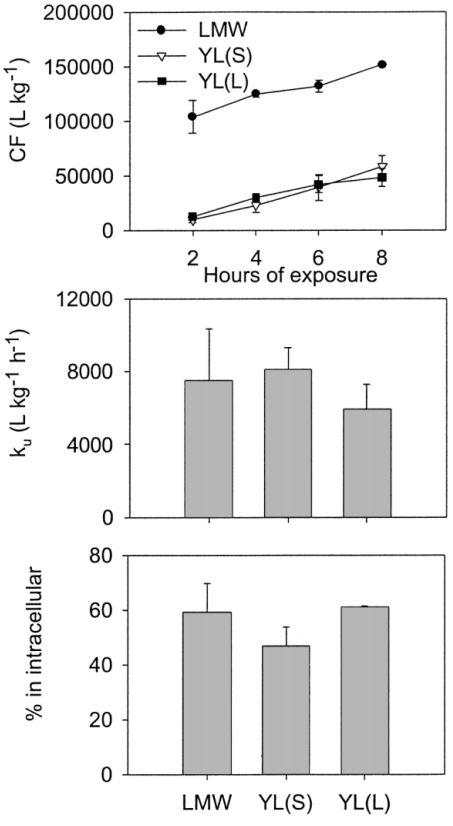


Fig. 4. Concentration factor (CF), uptake rate constant ($k_u$), and % in intracellular pool of $^{59}$Fe bound with different sizes of natural colloids isolated from estuarine waters (Yuen Long, YL) in *Trichodesmium*. LMW: Fe complexed with low molecular weight fraction (<1 kDa), YL(S): Yuen Long small colloids (1 to 10 kDa), YL(L): Yuen Long large colloids (10 kDa to 0.2 µm). Values are means ± SD (n = 3)

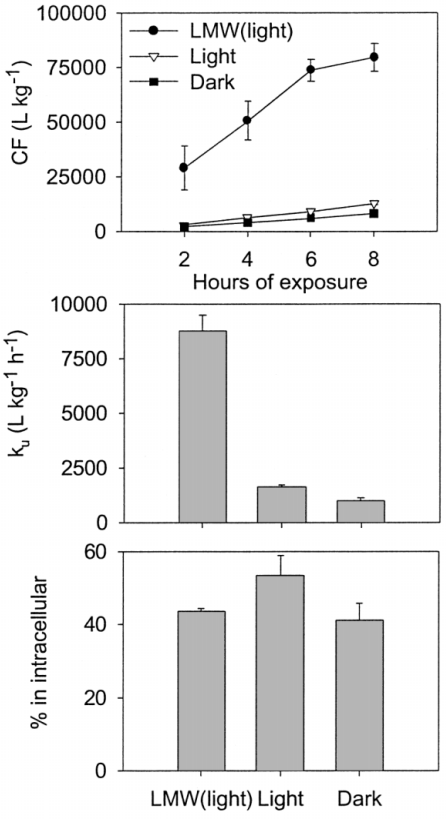


Fig. 5. Concentration factor (CF), uptake rate constant ($k_u$), and % in intracellular pool of $^{59}$Fe bound with Yuen Long natural colloids under light and dark conditions in *Trichodesmium*. LMW: Fe complexed with low molecular weight fraction (<1 kDa). Values are means ± SD (n = 3)

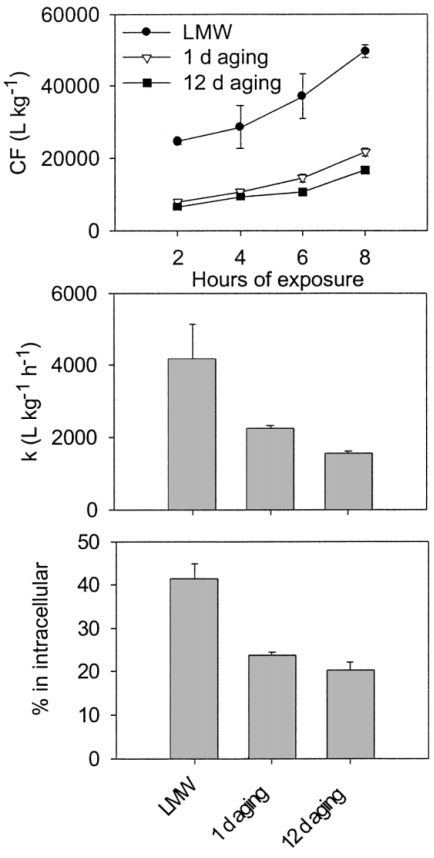


Fig. 6. Concentration factor (CF), uptake rate constant ($k_u$), and % in intracellular pool, of $^{59}$Fe bound with Yuen Long natural colloids for 1 and 12 d in *Synechococcus*. LMW: Fe complexed with low molecular weight fraction (<1 kDa). Values are means ± SD (n = 3)

### Fe uptake at different colloidal Fe concentrations

The calculated CFs in *Synechococcus* were somewhat comparable among the low colloidal Fe concentration treatments (38, 81, and 159 nM), and these were much higher (up to 12.2×) than the CFs of the 2 highest colloidal Fe concentration treatments (549 and 777 nM) (Fig. 7). At colloidal Fe concentrations <159 nM, there was no significant difference in the calculated $k_u$ (1-way ANOVA), suggesting that it was independent of the colloidal Fe concentration. At a much higher colloidal Fe concentration (>549 nM), however, the calculated $k_u$ was very low. For example, it was 4.3 to 7.7× lower at 549 nM than at the lower colloidal Fe concentrations. There was no apparent net uptake of colloidal Fe at 777 nM, and the $k_u$ was zero. The distribution of colloidal Fe in the intracellular compartment was much lower at the higher colloidal Fe concentration treatments than at the lower colloidal Fe concentration treatments, indicating that there was no biological uptake of colloidal Fe when the Fe concentration was much higher (777 nM).

## DISCUSSION

In this study, the biological availability of colloidal Fe was quantified as intracellular Fe accumulation in cells, representing the true Fe biological acquisitions. The radiotracer technique was employed to spike the natural colloids or produce the 'uniformly' radiolabeled phytocolloids. One premise in the use of the radiotracer technique is that the $^{59}$Fe behaved in the same way as the unlabeled Fe that the natural colloids contain. The natural colloids were radiolabeled with $^{59}$Fe for 2 d before the radioactive uptake experiments. It remains to be determined whether $^{59}$Fe was in full equilibrium with stable Fe in the colloids within 2 d of radiolabeling. Chen & Wang (2001) discussed the possibility that if $^{59}$Fe was not in true equilibrium (e.g. uniformly radiolabeled), it would lead to a more labile form than natural Fe, and thus the bioavailability may be an overestimation. There is a need to examine the exchange kinetics between Fe bound inside the colloidal matrix and the newly added radiotracer.

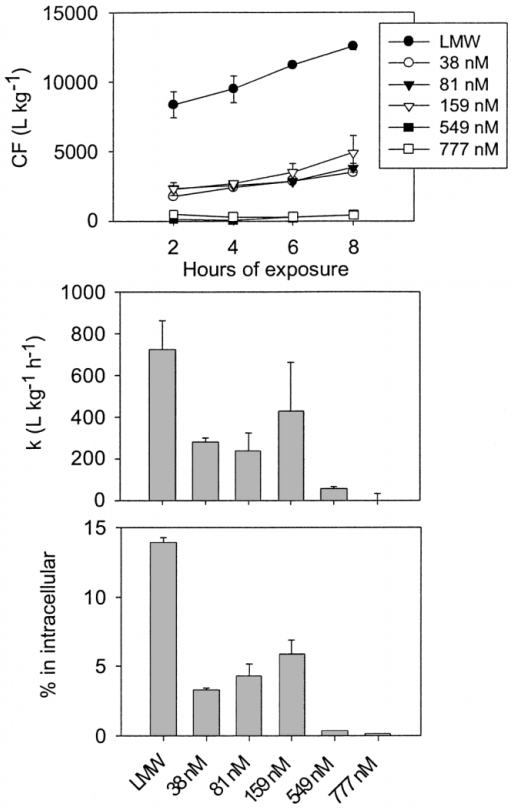


Fig. 7. Concentration factor (CF), uptake rate constant ($k_u$), and % in intracellular pool, of $^{59}$Fe bound with Yuen Long colloids at different colloidal Fe concentrations in *Synechococcus*. LMW: Fe complexed with low molecular weight fraction (<1 kDa). Values are means ± SD (n = 3)

Our study demonstrates that Fe bound with natural colloids was bioavailable to the 2 marine cyanobacteria, but such bioavailability was greatly dependent on the geochemical properties of the colloidal particles. In general, bioavailability of the colloidal Fe was much lower (>1.7× in all experiments) than the uptake of LMW-bound Fe. A much smaller fraction of cellular Fe was also found in the intracellular compartment as compared to the LMW treatments. To our knowledge, no previous study has examined the biological uptake of natural colloidal Fe by marine cyanobacteria. There were also few studies that examined the biological uptake of Fe bound with organically complexed ligands by cyanobacteria (Hutchins et al. 1999, Boye & van der Berg 2000). Boye & van der Berg (2000) suggested that *Emiliania huxleyi* was able to release Fe-complexing ligands in response to Fe addition. This organically complexed Fe was, however, not immediately available to *E. huxleyi*, and it appeared to only respond to freshly added inorganic Fe.

The actual uptake rate of the colloidal Fe by the cells can be directly calculated from $k_u$ and the colloidal Fe concentration in the bulk solution (Eq. 1). It is possible to compare our calculated cellular Fe uptake rate by the cyanobacteria with the previous literature measurements. Hutchins et al. (1999) examined the uptake of Fe complexed with different organic ligands by 2 species of cyanobacteria, *Synechococcus* CCMP 1334 and *Synechococcus* PCC 7002. The uptake rates of inorganic Fe and the siderophore-complexed Fe were in the range of 3.8 to $9.4 \times 10^{-21}$ mol cell$^{-1}$ h$^{-1}$ and 2.7 to $4.4 \times 10^{-21}$ mol cell$^{-1}$ h$^{-1}$ for *Synechococcus* CCMP 1334 and *Synechococcus* PCC 7002, respectively (Hutchins et al. 1999). Using Eq. (1), we calculated that Fe uptake rates by *Synechococcus* in our study were 2.4 to $32 \times 10^{-21}$ mol cell$^{-1}$ h$^{-1}$ and 1.3 to $9.5 \times 10^{-21}$ mol cell$^{-1}$ h$^{-1}$ for the LMW Fe and the estuarine colloidal Fe, respectively. These calculations were rather comparable to the measurements made by Hutchins et al. (1999) for the inorganic Fe and Fe complexed with siderophores. The uptake rate for the decomposed cyanobacterial colloids was $11 \times 10^{-21}$ mol cell$^{-1}$ h$^{-1}$. Furthermore, it may be possible to compare the total cellular Fe requirement with the Fe quota (as a result of colloidal Fe uptake) if uptake and cell growth rates are known.

It is relatively well known that marine cyanobacteria are able to produce siderophores which are released into the environment when the cells are under Fe-limited conditions, but the types of siderophores vary among the different species of cyanobacteria (Trick & Kerry 1992, Lewis et al. 1995). Trick & Kerry (1992) found that siderophores produced by *Synechococcus* sp. PCC 7942 had a distinct molecular size of 310 to 313 Da. The activation of a siderophore high-affinity transport system is important in the biological acquisition of Fe, allowing cyanobacteria to exist at low Fe environments (Wilhelm 1995). Wilhelm et al. (1996) demonstrated that *Synechococcus* under Fe-limited conditions was able to establish a growth rate comparable to that of the cells grown in Fe-replete conditions by releasing an extracellular siderophore, which facilitated the acquisition of Fe. In addition to the siderophore transport system, our present study also showed that cyanobacteria are able to acquire colloidal Fe from the ambient environment. The relative importance of the siderophore transport system and colloidal uptake pathway, however, remains to be further revealed.

In our study, we compared the uptake rate constant ($k_u$) and the concentration factor among different experimental treatments. An important consideration in colloidal Fe uptake studies when using different sources of colloids is whether the different Fe concentrations affect the interpretation of the bioaccumulation data. Our data demonstrated that $k_u$ was independent of the colloidal Fe concentration typically found in marine systems. Thus, $k_u$ can be directly compared

among different experimental treatments to examine the influence of different geochemical properties of colloids on the Fe uptake. Consistently, we observed no difference in $k_u$ at 2 different COC concentrations, further implying that the ambient Fe concentration did not affect $k_u$ at relevant Fe concentrations. At a much higher colloidal Fe concentration (>159 nM), the uptake of colloidal Fe (which equals $k_u$ multiplied by the colloidal Fe concentration in the bulk solution) decreased.

It should be recognized that although the biological uptake of colloid-bound Fe was lower than the uptake of LMW-complexed Fe, colloidal Fe may still provide an important source for Fe acquisition in the ocean, given its dominance over the free or inorganic Fe species in natural seawater (Wen et al. 1999, Wells et al. 2000, Wu et al. 2001). The biological significance of colloidal Fe may potentially be greater than the inorganic complexed Fe species, although the geochemical properties may largely affect the biological uptake of colloidal Fe. In addition, we examined only the Fe-repleted cyanobacteria in this study, and the ability of phytoplankton to acquire colloidal Fe may be greatly enhanced under Fe-limiting conditions.

Our study unequivocally showed that Fe bound with estuarine and decomposed cyanobacterial colloids was bioavailable to cyanobacteria, whereas colloids isolated from coastal waters and decomposed diatoms were essentially not bioavailable. The $k_u$ values of Fe bound with these estuarine and decomposed cyanobacterial colloids were only 1.7 to 3.8× lower than LMW-complexed Fe. Our study, therefore, highlights the specificity of colloidal Fe uptake by cyanobacteria. Colloids isolated from the semi-enclosed harbor water (Tolo Harbor) showed a somewhat intermediate uptake by the cyanobacteria. The mechanisms underlying such a difference were not exactly known because the chemical signatures of the colloids isolated from these regions were not quantified in our study. Colloidal particles isolated from the estuarine waters may be largely of terrestrial origins, with high concentrations of humic or fulvic substances, whereas colloids isolated from the coastal waters may be largely of biological activity such as phytoplankton decomposition. The phytoplankton community in Clear Water Bay was dominated by diatoms and dinoflagellates, with occasional blooms of cyanobacteria *Trichodesmium* (R. C. H. Dei & W.-X. Wang unpubl.).

In contrast to cyanobacteria, we recently found that the uptake of colloidal Fe by the marine diatom *Thalassiosira pseudonana* was much higher for colloids isolated from Clear Water Bay or coastal waters, whereas there was essentially no uptake of Fe bound with colloids from Yuen Long (Chen et al. 2003). These data strongly suggested that there is a clear difference in the biological acquisition of Fe bound with estuarine and coastal colloids between diatoms and cyanobacteria. Consistently, Fe uptake from colloids isolated from biological decomposition was dependent on phytoplankton species. Colloids from diatom decomposition showed a much lower uptake than colloids isolated from cyanobacterial decomposition. Our data are therefore consistent with the study by Hutchins et al. (1999), who demonstrated that there is a strong contrast between prokaryotes and eukaryotes in their biological uptake of organically complexed Fe. For example, they showed that eukaryotic phytoplankton efficiently assimilated porphyrin-complexed Fe, but this source was relatively unavailable to cyanobacteria. Conversely, Fe bound to a variety of siderophores was relatively more available to cyanobacteria than to eukaryotes, suggesting that the 2 plankton groups exhibited different Fe-uptake strategies. In contrast, Soria-Dengg et al. (2001) recently demonstrated that siderophores produced by bacteria can facilitate Fe uptake in the marine diatom *Phaeodactylum tricornutum*. In this study, we did not quantify the uptake of biologically decomposed colloids by diatoms.

The Fe concentration factor and the uptake rate constant varied among the different experiments due to differences in the physiological condition of cells. In each experiment, we used the LMW-complexed Fe to compare the relative uptake of colloidal Fe by the cyanobacteria. The uptake of Fe from decomposed *Synechococcus* colliods was 1.7× lower than the uptake of LMW-complexed Fe, as compared to 3.3– 3.8× and 50–60× lower uptake of Fe from YL and CWB colloids. It thus appears that the Fe bound with decomposed *Synechococcus* colloids was even more bioavailable than the estuarine colloids. In natural seawater, biological decomposition may therefore provide an important source for Fe to the cyanobacteria.

One possibility in our colloidal uptake experiment is that colloidal Fe may redistribute from the radiolabeled colloidal nanoparticles into the truly dissolved uptake medium. This is a potential problem in all uptake studies using the spiked radiotracer technique, and in fact presents a major obstacle when studying the biological availability of colloidal bound metals to aquatic organisms (Wang & Guo 2000). Among the many trace metals examined thus far (Cd, Cr, Fe, and Zn, Pb, Ag) (Carvalho et al. 1999, Wang & Guo 2000, Chen & Wang 2001, Guo et al. 2002), Fe probably has the strongest binding affinity with the colloidal particles. To overcome this difficulty, we used dialysis several times to ensure that all weakly bound Fe was removed before the uptake experiments. Consistently, the majority of the colloidal Fe (>93%) was associated with the colloidal phase in the controlled treatment without algal additions, which was also found in several previous

studies (Chen & Wang 2001, Guo et al. 2002, Chen et al. 2003). Evidence also indicates that repartitioning, if any, into bulk solution was unlikely to contribute significantly to the overall colloidal Fe uptake by cyanobacteria (M. Chen & W.-X. Wang unpubl. data).

In an earlier study (Chen & Wang 2001), we demonstrated that colloidal size and aging had a significant influence on the uptake of colloidal Fe by the diatom *Thalassiosira pseudonana*. Fe bound with larger colloids or those aged for a longer period exhibited lower diatom uptake. Our study on *Synechococcus* and *Trichodesmium* was generally consistent with this previous study, especially for colloids isolated from estuarine water with a relatively high uptake as compared to colloids isolated from the semi-enclosed harbor. Such a trend was observed in both species of cyanobacteria. These experimental results may help in the interpretation of the uptake mechanisms of colloidal Fe by cyanobacteria.

Several mechanisms have now been proposed for the biological uptake of Fe, such as photolysis (Waite & Morel 1984, Wells et al. 1991b, Johnson et al. 1994, Barbeau et al. 2001), biological reduction (Price & Morel 1998, Maldonado & Price 1999, 2000), and grazing regeneration (Barbeau et al. 1996, Barbeau & Moffett 2000). They all assume that inorganic or free-ion species [Fe(II) and Fe(III)] are the ultimate species taken up by marine phytoplankton, and organically complexed Fe must be converted into inorganic or free Fe species by photoreduction or biological reduction before Fe transport across the biological membrane can occur. Our experimental data were consistent with these transport mechanisms. Given the relatively high uptake of smaller and younger colloids, dissociation from the colloidal particles is presumably also the critical step for biological transport of colloidal Fe by cyanobacteria. Such transport is also consistent with faster uptake of LMW-bound Fe than colloidal Fe. However, such dissociation is most likely to occur at the cell surface instead of in the bulk seawater solution. The lower intracellular distribution of Fe in colloidal Fe treatments, as compared to the LMW treatment, further demonstrates the importance of Fe dissociation from colloids before cellular transport can occur. In marine phytoplankton, it has been postulated that biological reduction by reductase may be important for the acquisition of organically complexed Fe (Guerinot 1994, Maldonado & Price 1999, 2000). Consequently, biological reduction and chemical dissociation may be involved in the acquisition of colloidal Fe by cyanobacteria, in additional to the siderophore transport system (Hutchins et al. 1999).

Photolysis has been shown to be critical in the formation of lower-affinity Fe(III) ligands from Fe(III) complexes and the reduction of Fe(III), leading to an increase in the availability of Fe (Rich & Morel 1990). In general, we observed that the uptake of colloidal Fe and LMW-bound Fe was greater under light than dark conditions, presumably caused by a higher biological demand from phytoplankton, and the photolysis of colloidal Fe. Rich & Model (1990) observed that photochemistry was important in the conversion of Fe species, even at relatively low light fluxes using fluorescent lighting. To unequivocally demonstrate the significance of photoreduction, more carefully controlled experiments are required. In natural environments, where photoreduction may be critical for Fe uptake, it is therefore possible that the uptake of colloidal Fe by marine phytoplankton may be much higher than the rate we have measured under laboratory conditions.

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